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To,

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Through,

1. Prof. M. Jayashankar, Research Guide
   and
2. The chairman
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Respected Sir,

Sub: Submission of Final Synopsis – reg

Ref: 1. Registration Letter No.MU/EXB/Ph.D/CR.56/Micro./2015-16/E.13, Dated: 19.01.2016
2. Pre thesis submission colloquium held on 13/02/2019
3. Permission letter No.MU/EXB/Ph.D/CR.56/Micro./2015-16/E.13, Dated: 23.02.2019

With reference to the above subject and ref 3, I am submitting 12 copies of Final Synopsis of research work carried out by me on the topic entitled "Performance of Lignolytic Fungi from Kodagu in Decomposition of Agroresidues" for your kind perusal and further needful action.

Thanking you.

Date: 01.4.2019 yours sincerely
Encl: 12 copies of synopsis

Geethanjali P.A

Signature of the Guide:

Forwarded to the Registrar (Evaluation), MUM for further action

FINAL SYNOPSIS OF THE THESIS

ENTITLED

"PERFORMANCE OF LIGNOLYTIC FUNGI FROM KODAGU IN DECOMPOSITION OF AGRORESIDUES"

To be submitted to

Mangalore University

For the award of the degree of

DOCTOR OF PHILOSOPHY
IN
MICROBIOLOGY

By

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PERFORMANCE OF LIGNOLYTIC FUNGI FROM KODAGU IN DECOMPOSITION OF AGRORESIDUES

1. Introduction

The annual input of litter from the forests of Kodagu (D) is very high and it supports innumerable variety of microorganisms. The fungal flora of the Western Ghats, a hot spot of biodiversity reveals that forests in Kodagu have a rich and most diverse species composition. The study involves screening of fungi producing ligninase enzyme from the litter. This helps in documenting the lignolytic fungal diversity of Kodagu district. Synthetic and bacterial enzymes are being replaced by fungal enzymes in various industries. Therefore, it is highly significant at this stage to concentrate on widely distributed fungi for producing Ligninase enzymes of industrial importance.

The study also involves the usage of common agricultural wastes of the district for culturing of fungi. Coffee husk, Areca husk, Paddy straw, Banana stem waste, effluents from coffee pulping etc can be used for mass culturing of ligninase producing fungi. This process further helps in degrading these resistant agricultural wastes which is otherwise a global problem of concern. Thus, the efficient, sustainable decomposition and reusing of these agro residues is an ecological need as well as, in a nation like India, is economical too.

Considering the rich diversity of the Western Ghats of Kodagu,the present investigation was undertaken to isolate, identify and study the lignolytic enzyme activity of fungi in degradation of few agro-wastes which forms the pioneering work for this region. Clearly there is a substantial scope for experimental investigations among this group of fungi, the results of which may find application in various industries.

The ligno-cellulosic biomass is the most abundant and also the most renewable biomaterial on earth. The crop residues produced in agriculture amounts to be about 620 million tons in India (Pandey et al, 2009). A large portion of these agro-residues generated in this manner are generally used as roofing material, animal feed, fuel and as packing material, while the other half is discarded by burning in the field. Improper disposal practices often lead to air pollution reduces the abundance and activity if soil microorganisms and lead to soil erosion (Walia et al, 1999). Thus, the reusing of these agro residues is an ecological need as well as, in a nation like India, is economical too (Gaur, 1999).

Lignocellulolytic microorganisms found abundantly in soil can be used to degrade and recycle these residues which are rich in lignocellulosic compounds. The composted material

enhances soil fertility and improves soil health. These microorganisms possess enzyme systems to attack, depolymerize and degrade the polymers in lignocellulosic substrates. The lignocellulolytic fungi have proved extremely useful in delignification of lignocellulosic biomass (Sahni and Phutela, 2013).

Lignin biodegradation performed by fungi is a multistep process mediated by enzymes of the lignolytic complex. In order to depolymerize and mineralize lignin, the most efficient lignin degraders have developed an oxidative and unspecific system including extracellular enzymes (Erden, et al., 2009).Lignolytic enzymes have received much attention from researchers in last decades due to their ability to oxidise both phenolic and non-phenolic lignin related compounds as well as highly recalcitrant environmental pollutants, which makes them very useful for their application to several biotechnological processes.

The present study was undertaken to isolate and screen lignin degrading fungi from plantations and agricultural litter samples of Kodagu district, optimize the fermentation conditions for producing ligninase enzymes of industrial importance and to degrade some common agro wastes such as coffee husk, paddy straw and areca husk using potential isolated fungi. The output of this study would contribute to the exploration and exploitation of natural resources for commercial applications and energy conservations.

Objectives of the study

- Isolation and screening of the lignolytic fungi.
- Identification of the lignolytic fungi based on morphological and molecular techniques.
- Optimization of growth conditions for lignolytic enzyme production.
- Working out of the efficiency of lignolytic fungi in decomposing few agro wastes (C:N Ratio)

The above objectives have been studied and thesis thus developed based on the results obtained is discussed under the following chapters.

1. General Introduction.

2. Review of literature.

3. Isolation and screening of the lignolytic fungi

4. Identification of the lignolytic fungi based on morphological and molecular techniques.

5. **Optimization of growth conditions for lignolytic enzyme production**

6. Efficiency of potential lignolytic fungi to decompose coffee husk, paddy straw and areca husk

7. Summary

8. Bibliography

CHAPTER: 1

INTRODUCTION

In this chapter, a brief introduction to the research study undertaken is presented, wherein the necessity of undertaking the work is justified and the aims and objectives of the study are specified.

CHAPTER: 2

REVIEW OF LITERATURE

An exhaustive review of the relevant aspects of distribution and classes of lignin degrading fungi and the production of extracellular enzyme during Lignin Degradation has been presented. Extensive applications of lignin degrading enzymes and cultivation of lignolytic fungi for ligninase production are reviewed. The environmental concern due to the unscientific disposal of agricultural wastes and application of lignin degrading fungi in the decomposition of different agro waste have been reviewed from the previous research studies

CHAPTER: 3

ISOLATION AND SCREENING OF THE LIGNOLYTIC FUNGI

This chapter deals with the isolation of fungal isolates from different localities of Kodagu District and screening the isolates for their lignolytic activity. A total of 58 lignolytic fungi were isolated from 25 samples collected from various sampling sites. Based on the intense reddish-brown halo zone production around the colony, 15 isolates were selected for further investigation.The 15 fungal isolates when subjected to screening by confirmatory test for lignolytic enzymes production, about 5 isolates produced intense clear zone and highest % decolourization in MEB supplemented with different polymeric dyes. The results obtained from these studies is interpreted and discussed with reference to earlier researches.

CHAPTER: 4

IDENTIFICATION OF THE LIGNOLYTIC FUNGI BASED ON MORPHOLOGICAL AND MOLECULAR TECHNIQUES

In this chapter, the isolates were examined macroscopically and microscopically. Based on their morphological characteristics the isolates were identified up to genus level as *Mucor sp, Fusarium sp1, Fusaium sp2, Fusarium sp3 and Ceriporiopsis* sp. The fiive potential

lignolytic fungal isolates were confirmed by the molecular identification of the DNA based analysis of the internal transcribed spacers (ITS) rDNA marker. The BLAST analyses performed confirmed the identity of the above isolates as as *Mucor circinelloides* GL1, *Fusarium oxysporum1* GL2, *Fusaium oxysporum2* GL3, *Fusarium verticillioides* GL5 *and Ceriporiopsis* sp. PA1. The sequences were deposited in NCBI Genbank with accession numbers MF458974, MF458975, MF458976, MF458977 and MG923001 respectively.

CHAPTER: 5

OPTIMIZATION OF GROWTH CONDITIONS FOR LIGNINASE ENZYME PRODUCTION

In this chapter, the efficiency of potential fungal isolates namely *Mucor circinelloides* GL1, *Fusarium oxysporum1* GL2, *Fusaium oxysporum2* GL3, *Fusarium verticillioides* GL5 *and Ceriporiopsis* sp. PA1 to produce ligninase enzymes was studied by optimizing the culture growth conditions. Different physical and cultural conditions such as pH, temperature, incubation time, carbon source and nitrogen source inducers, surfactants and substrates were optimized for high yield of enzymes. When optimized media was used for lignolytic enzyme production, *Mucor circinelloides* and *Fusarium verticillioides* were found to exhibit maximum Laccase, Mn Peroxidase and Li Peroxidase activity as compared to other isolates. Laccase enzyme produced by *M. Circinelloides* was purified. About 18.8% as the final yield of purified enzyme with specific activity of 6436.7 U/mg was obtained at the end of purification step. The molecular weight of the enzyme was found to be 43 k Da. The results obtained from these studies is interpreted and discussed with reference to earlier researches.

CHAPTER: 6

EFFICIENCY OF POTENTIAL LIGNOLYTIC FUNGI TO DECOMPOSE COFFEE HUSK, PADDY STRAW AND ARECA HUSK

In this chapter, two isolates namely *Mucor circinelloides and Fusarium verticilloides* were studied for finding out their potential for the degradation of coffee husk, paddy straw and areca husk by estimating the pH, organic matter, organic carbon, total nitrogen, C: N ratio, lignin, holocellulose content, cellulase and ligninase activity of the inoculated and uninoculated substrates. The study showed that although both the isolates could degrade the agrowastes, *M. circinelloides* was a better lignolytic fungi as compared to *F. Verticillioides.* This is evident from the results obtained. Significant reduction of in lignin content, organic matter, organic carbon and holocellulose content, C:N ratio is recorded and the percentage reduction is

prominently higher when the substrates were degrade by *M. circinelloides* as compared to *F. Verticillioides.* During the course of degradation by lignolytic fungi, lignolytic enzyme activity and cellulase activity has been observed.

Note: For all the chapters, the results obtained are discussed in detail with relevant literature, suitable illustrations, figures and graphs in thesis.

CHAPTER: 7

SUMMARY

The summary and conclusion is discussed in detail in this chapter. The present study aimed at isolating and identification at the molecular level of lignin degrading fungi has thus generated information of lignolytic fungi in terms of optimum growth requirements, characterization of the enzymes produced by them and agro waste degradation potential of isolates. Thus, the present research is an important step towards understanding the potential of these lignolytic fungi for their further exploitation for vast biotechnological applications.

CHAPTER: 8

BIBILIOGRAPHY

The references cited in the thesis are listed alphabetically as per the conveniently accepted citation pattern in this chapter.

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Four publications in journals, two in conference proceedings and eleven research papers presented in national and international seminars/conferences related to this thesis

Signature of Guide

Signature of Research scholar